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The diagnosis and management of a case of leishmaniosis in a dog imported to Australia

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\textbf{Abstract}

This case study discusses in detail for the first time the diagnosis and management of a case of leishmaniosis in a dog imported to Australia. The dog presented with epistaxis and a non-regenerative anaemia five years after being imported from Europe. Protozoa were identified within macrophages in bone marrow and splenic cytology. A \textit{Leishmania} indirect fluorescent antibody test was performed and was positive while an \textit{Ehrlichia canis} antibody test was negative. Polymerase chain reaction of the ITS-1 and ITS-2 regions of skin, lymph node,
spleen and bone marrow were all positive for *Leishmania infantum*. The dog was treated with amphotericin B with a strong clinical response. The importance of thorough diagnostics in non-endemic areas, particularly Australia, is discussed. Treatment with amphotericin B is discussed. Vigilance, disease reporting and response frameworks are recommended for non-endemic areas.

**Keywords:** Australia; Amphotericin B; Dog; *Leishmania infantum*; PCR

**Introduction**

Canine leishmaniosis is a zoonotic tropical disease with a widespread global distribution (Solano-Gallego et al., 2009) and an expanding geographical range (Gonzalez et al., 2010 and Gramiccia, 2011). It is a significant zoonotic disease with World Health Organisation global burden disease estimates placing *Leishmania* second for mortality and fourth for morbidity amongst human tropical diseases (Mathers et al., 2007). Diagnostic options for canine leishmaniosis have increased in number and accuracy in recent years but definitive diagnosis in dogs still requires several, concordant test results (Solano-Gallego et al., 2009 and Gramiccia, 2011). Opinions on optimal and ethical treatment vary with compulsory culling still current in some countries, whereas increasingly sophisticated treatments are employed elsewhere (Gramiccia, 2011 and Dantas-Torres et al., 2012). While some question the ability to truly cure canine leishmaniosis (Ribeiro et al., 2008, Manna et al., 2009 and Torres et al., 2011) clinical cures can be achieved with some dogs no longer infectious through their biological vectors (da Silva et al., 2012).
In Australia there is no recognised endemic zoonotic Leishmania species present. A small number of cases have been recorded in imported dogs but to date none of these have been published. An endemic Australian Leishmania species in a captive population of red kangaroos (Macopus rufus) (Rose et al., 2004) and in several species of wild macropods (Dougall et al., 2009) has been discovered recently. This creates a need for rigorous diagnostic testing to identify the causative species if leishmaniosis is suspected in Australia. While phlebotomine sandflies are present in Australia there are no known competent biological vectors of the non-Australian Leishmania species present. A day-flying midge, species Forcipomyia (Lasiohelea) spp., has been implicated as the vector for endemic Australian Leishmania (Dougall et al., 2011) and this is the first non-sandfly insect strongly implicated as a natural vector of any Leishmania species. In light of these findings a thorough disease plan should be in place for suspected cases of Leishmania in Australia; including comprehensive diagnostic testing, clear treatment goals, monitoring of response to treatment and measures to minimise the risk of transmission to other dogs and people. While Australia is in a unique situation with regards to Leishmania, many considerations are likely applicable to other extralimital areas.

**Case report**

A nine year old female neutered husky dog was presented with a three month history of bilateral mucopurulent nasal discharge, which had progressed to minor epistaxis three weeks prior to presentation, and major epistaxis three days prior to presentation. Previous medical history included three years of presumed atopic dermatitis which had been treated with courses of prednisolone and cephalexin. The dog had recently developed bilateral symmetrical alopecia and calcinosis cutis, attempts to withdraw the prednisolone had resulted
in collapse 48 h later. The dog was born in Portugal and had lived in the UK, during which
time the dog had travelled back and forth from Portugal to the UK. In 2007 at the age of five
the dog was emigrated to Australia and, after the required period of quarantine, had moved to
a Brisbane suburb and resided there for the last four years, with no history of wider travel
within Australia. Serological testing for *Leishmania* via IFAT or ELISA was required prior
to import to Australia at this time. The precise test information for this dog is not known but
presumably the result was negative.

Clinical examination revealed no obvious structural, pigmentation or sensation changes
around the nasal cavity with normal symmetrical airflow. The mucous membranes were pale
with a rapid refill. There was widespread non-pruritic symmetrical alopecia with calcinosis
cutis and small open sores. Hepatomegaly and mild peripheral lymphadenopathy were
appreciated.

An abdominal ultrasound showed a large hyperechoic liver containing a cavitated and
partially mineralised mass, suspected to be a benign lesion, while the adrenal glands were
small and inconspicuous. A biochemistry panel was performed (Chem17 + lytes4, Catalyst
Dx; IDEXX) which revealed a hepatopathy with an ALP 1514 U/L (reference interval (RI):
23–212 U/L), ALT 143 U/L (RI: 10–100 U/L), GGT 42 U/L (RI: 0–7 U/L), a mild
hyperglobulinemia at 46 g/L (RI: 25–45 g/L) and a slightly decreased creatinine at 42 μmol/L
(RI: 44–159 μmol/L). An activated clotting time (VetScan I-STAT; Abaxis) was 106 s (RI:
90–130 s). The PCV was 15% (RI: 35–57%) while a smear examination suggested a non-
regenerative anaemia with morphologically normal red and white cells. A single macrophage
at the feathered edge of a blood smear was observed with suspected intraerythroplasmic
organisms. Cystocentesis was performed and the urine had a specific gravity of 1.013 with an active sediment from which multisensitive *Escherichia coli* was isolated.

The dog was sedated for bone marrow aspiration and a nasal computed tomograph. Bone marrow cytology showed adequate populations of all cell-line precursors with approximately 10% of cells present being macrophages with the cytoplasm distended by populations of protozoa with 2 μm diameters, a dark nucleus, a single bar-shaped kinetoplast and pale-blue cytoplasm. Nasal CT was unremarkable with no mass effect or erosive process detected.

Following notification of and discussion with the Department of Agriculture, Forestry and Fisheries Queensland, extensive sampling was performed to allow confirmation of the diagnosis prior to treatment. This included collection of multiple aliquots of EDTA blood and serum, skin punch biopsies, skin scrapes, thick and thin smears of splenic aspirates and lymph node core biopsies. These samples were tested at the Australian Animal Health Laboratory and Murdoch University. Protozoal organisms were identified on cytology of the bone marrow and in the splenic aspirates (Fig. 1). Histology of the skin and lymph node did not identify any organisms. A commercially available (VRMD, 2011) *Leishmania infantum* indirect fluorescent antibody test (IFAT), considered genus specific (OIE, 2008), was performed and was positive at 1:6400 and negative at 1:12,800 indicating a high *Leishmania* antibody titre. An *Ehrlichia canis* antibody test (Protatek immunofluorescence antibody slide test) was negative. PCR analysis using the ribosomal ITS-1 and ITS-2 regions (Tai et al., 2000) was performed on the skin, popliteal lymph node, peripheral blood and bone marrow samples. All samples were positive by PCR and sequenced nucleotides were found to be a 100% match with published sequences of *L. infantum* (NCBI Accession numbers AJ634355/AJ634339).
The affected dog had three companions that closely cohabited in an outdoors run with close contact during the day and night with biting insects known to be present. All three companion dogs, two huskies and a malamute, were negative on *L. infantum* IFAT.

At 28 days following initial presentation treatment was begun and a deltamethrin-impregnated collar was obtained (Scalibor; Intervet). Amphotericin B lipid complex was given intravenously at 50 mg per dose (approximately 2.5 mg/kg) diluted to 1 mg/ml in 5% dextrose in water and given over one hour. Creatinine, ALT and electrolytes were monitored, a 10 ml/kg fluid bolus was given and maropitant (Cerenia; Zoetis) was administered prior to each treatment. An acute increase in ALT up to 702 U/L was recorded following the second treatment with normal bile acid stimulation test results. This reduced rapidly with supportive care including SAMe (Denosyl; Ceva) and a short treatment delay. Following the fourth dose acute kidney injury was indicated by a creatinine of 872 μmol/L, resulting in seven days hospitalisation on fluid therapy prior to discharge for home care with an esophagostomy tube placed. Further treatment was deferred for 53 days after which time creatinine was back within normal limits and treatment was resumed with a similar protocol to the initial round of treatment, except daily doses of 25 mg amphotericin B were given for four consecutive days. This treatment was tolerated well with a moderate increase in ALT to 384 U/L observed but no renal compromise or clinically apparent side effects noted. The total dose of amphotericin B given was approximately 15 mg/kg.

One month following completion of the treatment blood PCR was negative for *L. infantum* and allopurinol was started at a dose of 15 mg/kg orally twice daily.
Discussion

Leishmaniosis presents a major diagnostic and management challenge wherever it occurs. In endemic areas the approach to this disease varies widely and remains contentious (Dantas-Torres et al., 2012). In non-endemic areas there are sporadic case reports of disease (Slappendel and Teske, 1999) but even where travel-related disease is relatively common, such as in the United Kingdom, the disease is often not closely monitored, is likely underreported and the risks of transmission are poorly understood (Shaw et al., 2009).

In this case thorough diagnostics excluded potential alternative infections, such as *E. canis* which can cross react serologically (Ferreira et al., 2007 and Marcondes et al., 2011), primarily through the use of PCR analysis. Appropriate testing will depend upon geographic location and should be conducted under the consultation or supervision of the relevant government authority as *Leishmania* is an OIE-listed disease (OIE, 2013). Monitoring of disease incidence allows protection of public health and response to changes in the disease prevalence or transmission. Where leishmaniosis is not a notifiable disease its prevalence is underreported (Shaw et al., 2009) and the risk of failing to detect a new or low density competent vector is increased.

The decision to treat is controversial. In this case treatment was performed due to the lack of known local vectors which made epidemic veterinary disease and zoonotic disease highly unlikely.
Amphotericin B is not recommended in endemic areas due to concerns about side effects and induction of human resistance. It was used in this case because of its excellent availability and low cost compared to other suitable agents in Australia, the low risk of development of resistance, the low likelihood of zoonotic transmission and the patients’ clinical suitability for this medication with a low serum creatinine prior to treatment.

Please consult the supplementary material provided electronically with this article for in depth discussion and review of the literature discussing the major issues of canine leishmaniosis in extralimital areas; including diagnosis, the decision to treat, the risks of transmission, treatment options and disease monitoring. The decisions made in this specific case are further discussed in this material.

In conclusion the increasing incidence of leishmaniosis in non-endemic areas is challenging owners, veterinarians and government authorities. These authors identify a need for clear, locally relevant guidelines directing diagnosis, treatment and management of suspected cases. This case report provides an example of such case management and highlights issues of particular relevance to Australia, such as the local status of *Leishmania*, incidence of canine travel to endemic areas, local climate and possible local vectors. These guidelines would be recommended for all governments in non-endemic *Leishmania* areas.

**Conflicts of interest**

The authors have no conflicts of interest concerning the work reported in this paper.
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References

References


Figure 1. Cytology of a splenic macrophage containing protozoal organisms within the cytoplasm. Organisms are approximately 2 μm in diameter, with a blue nucleus, a single dark purple kinetoplast and a pale blue cytoplasm. Bar = 10 μm.